dynamic structural model for the TCR αβ TM complex and how it associates with the CD3 ζζ subunit. Additionally, the interaction of the TCR with its lipid environment may also regulate the organization of the TCR-CD3 TM complex. We will also discuss simulation of the TCR TM region in complex lipid bilayers that resemble plasma membranes. Our results suggest preferential interactions of the TCR TM region with cholesterol and anionic lipids in the bilayer.

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Evolutionary Avoidance of Transmembrane Embedded Arginines is due to Slowed Folding Kinetics

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Conserved transmembrane (TM) arginines are essential for the transport function of many membrane proteins, such as voltage-gated ion channels. However, among the lipid-facing residues in TM regions, the statistical propensity of arginines is extremely low. The minimal occurrence of TM arginines is often attributed to the thermodynamic cost of inserting an arginine into a hydrophobic lipid environment. But the energetic cost of TM arginine insertion measured by the Moon-Fleming Hydrophobicity scale is only 3 kcal mol-1, implying that Arg should be allowed in the bilayer. To investigate the discrepancy between this moderate thermodynamic cost and the evolutionary avoidance of TM arginines, we utilized Outer Membrane Phospholipase A (OmpLA) as a host for lipid-facing arginine mutations, at three different depths in the bilayer. We probed the effects of the introduction of TM arginines on the secondary structure and enzymatic activity of OmpLA and found that Arg has a minimal impact on the structure and function of OmpLA. We also determined that a TM Arg does not affect the thermodynamic binding of OmpLA to the periplasmic chaperones, SurA and Skp. Molecular dynamics simulations revealed that all TM Args studied form hydrogen bonds with water and phospholipid head groups. Kinetics of folding into large unilamellar vesicles of varying head group compositions reveal that the R-variants exhibit slow folding, even in the presence of the OMP assembly machinery protein, BamA. Slow kinetics are additionally observed for in vivo folding of these R-variants. In addition to a thermodynamic cost for TM arginine insertion our results indicate that a pronounced kinetic barrier may play a role in the evolutionary pressure against TM arginines.

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Site Directed Spin Label EPR Spectroscopy of Influenza a M2 Protein

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M2 is a membrane protein critical to the life cycle of influenza A. We have capitalized on the expanding body of biochemical and high-resolution structural data available for the M2 protein to design and interpret site-directed spin labeling electron paramagnetic resonance spectroscopy (SDSL-EPR) experiments on the conformation and dynamics of the homotetrameric M2 protein embedded in lipid bilayers. We have obtained data for three different M2 constructs (M2TM 22-46, M2TMC 23-60 and full length M2 protein) spin-labeled at multiple sites within the transmembrane and C-terminal domains. CW and pulsed EPR spectra show evidence that M2 adopts multiple conformational states in bilayers, and that cholesterol content dictates the relative populations of the states. Furthermore comparison of full-length M2 protein and a M2TMC 23-60 peptide demonstrates that the C-terminal juxtamembrane region in both constructs forms an amphipathic membrane surface helix.

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Studying the Membrane-Bound Conformation of Alpha-Synuclein using a Model Transmembrane Peptide System in a Lipid Bilayer

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Alpha-synuclein is intrinsically disordered in solution but adopts a partially alpha-helical structure when membrane bound; we are currently investigating this structure using a cyanylated cysteine vibrational probe at various sites on the αS membrane binding domain. In order to quantitatively understand the depth dependence of the SCN probe in a lipid bilayer, four variants of a poly-L transmembrane helical peptide were designed and synthesized with cysteine residues at different depths in a membrane bilayer. A nonaqueous cyanylation and purification protocol was developed to introduce the probe group on these very hydrophobic model peptides. Vibrational spectroscopy was carried out on the four variants in a model lipid system using both solution/vesicle and oriented, immobilized samples. These data will help to elucidate the membrane interactions of both αS and other membrane-bound proteins.

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Structural Comparison of Membrane-Bound Retroviral Gag Proteins

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he Gag polyprotein is essential for the assembly of retroviral particles. It is expressed in the cellular cytoplasm and targets the surface of the plasma membrane, where assembly occurs. All Gag proteins from various retroviral species contain structured domains - the membrane-binding matrix (MA), the capsid (CA) and the genome-binding nucleocapsid (NC) domains - that are separated by unstructured linker regions. Cryo-EM of immature virions shows assemblies on the plasma membrane in which Gag polyproteins are in an extended conformation, apparently stabilized by lateral interactions between their CA domains. However, the structural dynamics are complex. In a recent study with neutron reflection (NR), we showed that in HIV-1 Gag, the MA-CA linker is so flexible that the polyprotein binds a model membrane in a back-folded structure in the absence of nucleic acid, and that a transformation into an extended conformation is triggered upon nucleic acid binding (Datta et al., J. Mol. Biol. 406, 2011, 205). Here we investigate the membrane-binding of murine leukemia virus (MLV) and Rous sarcoma virus (RSV) Gag proteins, which also include unstructured linkers between their MA and CA domains. Both wild-type MLV and RSV Gag bind membranes in vitro in folded conformations, as shown by NR, indicating structural flexibility of their MA-CA linkers. In contrast, an MLV mutant, Δp12, in which the MA-CA linker has been eliminated, binds the membrane in an extended conformation. An investigation of the RSV mutant, Δ104-220, that is analogous to MLV-Δp12, is currently in progress. These studies suggest that the disordered linker regions in retroviral Gag proteins may function to prevent unproductive assembly processes in the host cytosol or on the membrane in the absence of viral genome.

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Probing Protein-Lipid Interactions at the Single Molecule Level

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Cell membranes consist mostly of lipids and embedded proteins, forming a shell around the cell that controls information and material fluxes into and out of the cell. Protein-lipid interactions thus play a crucial role in numerous cellular mechanisms. Understanding these interactions would provide basic biophysical knowledge in addition to advance pharmaceutical developments, many of which target membranes. Despite their significance, protein-lipid interactions are not fully understood and have not yet been fully investigated from a mechanistic standpoint. In this study we investigated synthesized peptide constructs that insert into lipid bilayers via force spectroscopy. The constructs used here are based on SecA, a membrane-associated ATPase of the general secretory system. We investigated the first 10 amino terminal residues of SecA (SecA2-11) in three different arrangements to provide control experiments and guide interpretation. In one arrangement, two copies of SecA2-11 were covalently linked in series to the atomic force microscope tip, in another, they were linked in parallel. In all cases the constructs were brought into close proximity of a supported POPC lipid bilayer and the mechanical interaction between the peptide and lipid was recorded using single-molecule dynamic force spectroscopy. Investigating chemically similar yet geometrically different constructs provides important benchmarks for the interpretation of future studies involving longer constructs such as full length SecA.

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Single-Molecule Diffusion Measurements Indicate Independent Membrane Insertion by the Tandem C2 Domains of Synaptotagmin 7

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The synaptotagmin (Syt) family of proteins is characterized by the presence of tandem C2 domains, C2A and C2B, which sense Ca^{2+} to trigger vesicle fusion during exocytosis. The widely studied Syt1 is central to rapid neurotransmitter release, while Syt7 is involved in slower secretion of large dense-core vesicles and has C2 domains that dock much more tightly to target membranes. Despite